REMARKS

Claims 1-11 have been canceled without prejudice or disclaimer. Applicants reserve the right to file one or more continuation or divisional applications directed to the canceled subject matter. Basis for the recitations "wherein said device generates a sufficient electrostatic charge to capture viable organisms on a grounded, conductive material" and "wherein said electrostatic field generates a sufficient electrostatic charge to capture viable organisms on a grounded, conductive plate" can be found, for example, in paragraphs [0036], [0046], [0048], and the examples. No new matter has been added. Entry and conderation are respectfully requested.

The objection to the drawings because of empty boxes (3) and (4) in Figure 3 is respectfully traversed.

Applicants have attached corrected Figure 3 in compliance with 37 CFR 1.121(d) as required by the Office in the above referenced Office action. Withdrawal of the instant objection is respectfully submitted.

The rejection of claims 1-5 and 7-11, as it now pertains to new claims 12-16 and 18-22, under 35 USC 102(b) as being anticipated by Mitchell et al is respectfully traversed.

Applicants submit that Mitchell et al. fail to teach an electrostatic sampling device wherein said device generates a sufficient electrostatic charge to capture viable organisms on a grounded, conductive material or wherein said electrostatic field generates a sufficient electrostatic charge to capture viable organisms on a grounded, conductive plate. Mitchell et al teach an electrostatic space charge system which has a ground plane which causes power requirements for the battery-powered high voltage supplies to be excessive since the closer a ground plane is brought to the discharge electrodes, the higher the load on the power supply. Futhermore, the system of Mitchell et al uses

operating voltages in the range of about -15,000 dc to about -30,000 dc which are levels known to be lethal to airborne and surface bacteria (See Arnold et al., J. Appl. Poult. Res, 179-186, 2002; enclosed and Seo et al., J. of Food Prot., Volume64(1), abstract; enclosed).

The Federal Circuit states that the anticipation determination is viewed from one of ordinary skill in the art and that there must be no difference between the claimed invention and the reference disclosure as viewed by a person of ordinary skill in the field of the invention, Scripps Clinic & Research Foundation v. Genentech Inc., 927 F. 2d 1565, 18 USPQ2d 1001, 1010, (Fed. Cir. 1991). Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. It is not enough, however, that the reference disclose all the claimed elements in isolation.

The rejection is improper.

Applicants respectfully request withdrawal of the instant rejection.

The rejection of claim 6, as it now pertains to new claim 17, under 35 USC 103(a) as being unpatentable over Mitchell et al in view of Spurrell is respectfully traversed.

Applicants respectfully submit that the combination of Mitchell et al in view of Spurrell fails to render the instantly claimed invention *prima facie* obvious. Mitchell et al. fails to teach a device which generates a sufficient electrostatic charge to capture viable organisms on a grounded, conductive material.

The Mitchell et al patent teaches a device which generates an operating voltage in the range of about -15,000 dc to about -30,000 dc which is lethal to organisms as stated above in the 102(b) rejection and herein incorporated by reference in its entirety.

Mitchell et al in view of Spurrell fails to cure the deficiency of Mitchell et al because Spurrell also fails to teach any type of electrostatic device and therefore fails to teach an electrostatic sampling device wherein said device generates a sufficient electrostatic charge to capture viable organisms on a grounded, conductive material or wherein said electrostatic field generates a sufficient electrostatic charge to capture viable organisms on a grounded, conductive plate. No other references have been provided to cure the deficiencies of the combination of Mitchell et al. in view of Spurrell. The rejection is improper. Applicants respectfully request withdrawal of the instant rejection.

It is believed that all of the claims are in condition for allowance. Accordingly, it is respectfully requested that the instant application be allowed to issue. If any issues remain to be resolved, the Examiner is invited to telephone the undersigned at the number below.

In the event this paper is deemed not timely filed, the undersigned hereby petitions for an appropriate extension of time. Please charge any fees, which may be required by this paper or at any time during prosecution of the instant application, or credit any overpayment, to deposit account 50-2134.

Respectfully Submitted,

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, Virginia 22313-1450, on July 6, 2005 by:

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Gail E. Poulos
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July 6, 2005
(DATE)

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Bactericidal Effects of Negative Air Ions on Airborne and Surface *Salmonella* Enteritidis from an Artificially Generated Aerosol

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ABSTRACT

The bactericidal effect of high levels of negative ions was studied using a custom-built electrostatic space charge device. To investigate whether the ion-enriched air exerted a bactericidal effect, an aerosol containing *Salmonella* Enteritidis (SE) was pumped into a sealed plastic chamber. Plates of XLT4 agar were attached to the walls, top, and bottom of the chamber and exposed to the aerosol for 3 h with and without the ionizer treatment. The plates were then removed from the chamber, incubated at 37°C for 24 h, and colonies were counted. An average of greater than 10^3 CFU/plate were observed on plates exposed to the aerosol without the ionizer treatment (control) compared with an average of less than 53 CFU/plate on the ionizer-treated plates. In another series of experiments, the SE aerosol was pumped for 3 h into an empty chamber containing only the ionizer and allowed to collect on the internal surfaces. The inside surfaces of the chamber were then rinsed with 100 ml phosphate-buffered saline that was then plated onto XLT4 plates. While the rinse from the control chamber contained colony counts greater than 400 CFU/ml of wash, no colonies were found in the rinse from the ionizer-treatment chamber. These results indicate that high levels of negative air ions can have a significant impact on the airborne microbial load, and that most of this effect is through direct killing of the organisms. This technology, which also causes significant reduction in airborne dust, has already been successfully applied for poultry hatching cabinets and caged layer rooms. Other potential applications include any enclosed space such as food processing areas, medical institutions, the workplace, and the home, where reduction of airborne and surface pathogens is desired.

Keywords: No keywords available.

Use of Negative Air Ionization for Reducing Microbial Contamination on Stainless Steel Surfaces

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Primary Audience: Poultry Processing Management, Quality Assurance Personnel, Researchers, Poultry Extension Specialists

SUMMARY

Microbiological concerns in food plant sanitation that relies heavily on physical and chemical methods for removing and killing bacteria could be reduced by the use of non-chemical intervention methods. This initial work on the effects of electrostatic space charge on biofilms shows promise as a viable intervention option for reducing bacterial contamination on surfaces. Natural bacterial populations from a poultry processing facility were collected, allowed to multiply and form biofilms, and assessed for susceptibility to negative air ionization. A small chamber with an electrostatic space charge system was used to treat the mixed bacterial populations that were grown on stainless steel coupons (1 × 4 cm). The object of the system was to transfer a strong negative electrostatic charge to bacteria that were attached to coupons at the base of the chamber. The system effectively decreased the survival levels of bacteria on the stainless steel, with a reduction efficiency of 99.8%. All of the swab samples taken from coupons were culture positive for bacteria, and the bacterial counts from the ionized surfaces were significantly less than for the non-ionized surfaces ($P <$ 0.01). These results demonstrate the potential efficacy of negative air ionization against bacterial contamination on surfaces in the poultry processing environment.

Key words: biofilm, negative air ionization, pathogen reduction, poultry, stainless steel

2002 J. Appl. Poult. Res. 11:179–186

DESCRIPTION OF PROBLEM

Bacterial contamination and biofilm formation on food contact surfaces in poultry processing facilities can lead to contamination of raw poultry products and potentially to food-borne illness for consumers. The primary approaches used to reduce the presence of bacterial pathogens and biofilms have included physical treatments, such as steam, water pressure, and heat treatments, as well as chemical treatments, such as chlorine, trisodium phosphate, and quaternary ammonium compounds [1]. Negative air ionization has been shown to be a promising new technology that is a safe, non-toxic, and non-chemical means of eliminating

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dust and pathogens from the air. The electrostatic space charge system (ESCS) approach has been shown to reduce airborne levels of *Salmonella enteritidis* (SE) by 95% in caged layer rooms [2] and to reduce airborne transmission by 98% in controlled-environment cabinets [3]. The mechanism, by which these reductions were achieved, was attributed primarily to the reduction in airborne dust levels [4, 5]. Most airborne bacteria are attached to dust particles [6]. The process by which the ESCS in poultry areas can remove air-polluting microorganisms involves charging airborne particles in the space and collecting them on special grounded collector plates or on the walls [7]. Ionization studies with SE suggest that a significant step toward producing SE-free eggs can be achieved by improving air quality in production houses where the eggs are produced [2]. This concept has been shown to work in filtered-air positive-pressure (FAPP) houses used to produce disease-free poultry [8, 9].

Air ionization can kill airborne and surface microorganisms. The potential effect of ions to destroy microorganisms has been suggested in numerous studies [10, 11, 12, 13]. Seo et al. [14] used an identical type of ESCS and test chamber as the one used in the present study to demonstrate that negative air ions could reduce aerosolized SE by 99% or more at close range. The minimum ion density dose and time exposure combinations required to kill organisms and the exact mechanisms that cause air ions to be bactericidal, however, have not been well established.

Hoenig et al. [15] stated that a corona discharge produces electrons, negative molecular ions, ozone, and ultraviolet light. Kellogg et al. [16] cautiously identified the hydrated superoxide radical anion $(O_2^-)(H_2O)_n$ as the negative air ions responsible for killing microorganisms. In another study [17], however, the result was not consistent. In spite of promising results reported, poor design of ion generators has resulted in low ion outputs or undesirable side products such as ozone. Studies on air ionization have been drawing a great deal of attention because of a variety of biological aspects ranging from lethal effects on microorganisms to therapeutic effects [18]. Ionization has enhanced treatment of seasonal affective disorder [19], chemotherapy [20], and respiratory nebulizers [21]. Following several years of development with a commercial company, a custom ESCS was developed by Mitchell and Stone [22], primarily for use in poultry areas to improve air quality by reducing airborne dust and microorganisms. This device generates very high ion density levels in large spaces and does not require air to move through it in order to be charged. Ozone production could not be detected at 0.01 ppm.

For this study a smaller, bench-top version of the ESCS has been developed. The primary objective was to kill bacteria in biofilms on stainless steel surfaces that could result in food spoilage or pose a threat to food safety.

MATERIALS AND METHODS

Apparatus Setup

A miniature ESCS was custom-built for this application (Figure 1). The ionizer consisted of an array of four ionizer bars 13 cm long, with seven pointed electrodes each, and a ground plane 7.6 cm above the electrode points. The bars were suspended 27.6 cm above the samples inside a closed chamber, 40.5 cm × 20.0 cm × 29.5 cm. The samples were placed on a grounded metal plate on the floor of the chamber. Ion densities at this distance were greater than $1 \times 10^6/cm^3$ (maximum measurable density by our instrumentation). Ionizer voltage was maintained at −25 kV by a Simco power supply during sample incubation. Air flow through the chamber was generated by an air pump [23] at 2 L/min, and humidity was maintained inside the chamber by passing the air from the pump through Tygon tubing (0.5-mm i.d.) to water inside a 2-L Erlenmeyer flask and then through tubing in the top of the flask to an opening in the chamber 14 cm above the samples.

Experimental Design

Biofilms were developed on stainless steel coupons by using a bacterial cocktail obtained from broiler carcass rinses. The steel used for the coupons in this study was 11-gauge (3.04 mm) stainless steel plate [24] with a 2B mill finish. Coupons (4 × 1 cm) were cut from the plate and divided into three groups for treatment by air ionization: a) treated coupons with

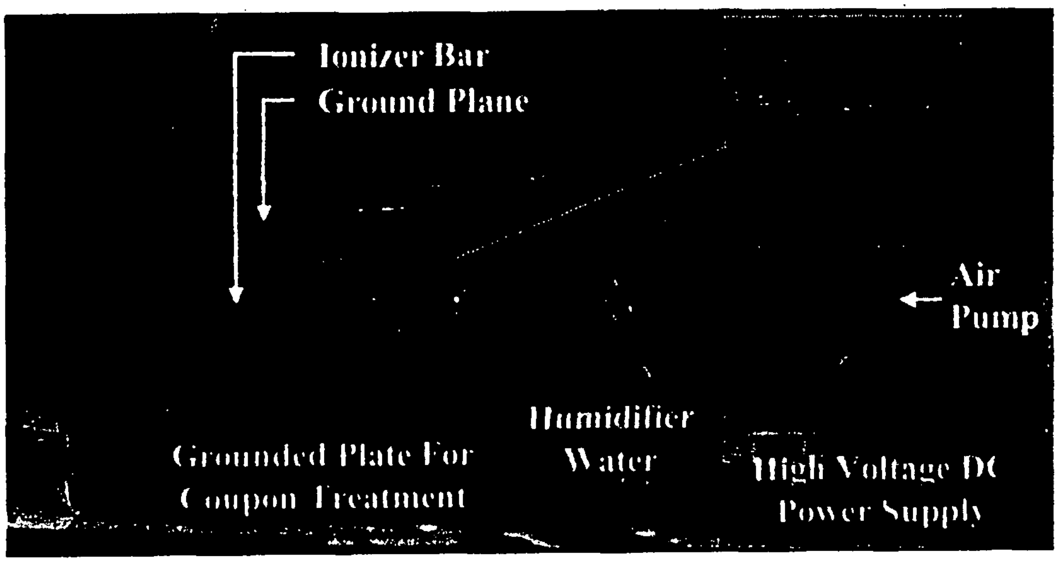

FIGURE 1. Equipment set up for negative air ionization system. The inset shows closeup of ionizer bars.

bacteria, b) untreated coupons with bacteria, and c) negative controls (untreated coupons without bacteria). To collect bacteria for growing biofilms, whole broiler carcasses were collected from a commercial poultry processing plant, bagged, weighed, and rinsed in 100 mL phosphate-buffered saline. The rinses were pooled, and 1-mL aliquots were serially diluted to measure bacterial colony-forming units (cfu) per milliliter. The rinse was frozen, and aliquots (200 μL) of the rinse were resuscitated by inoculation into 9 mL trypticase soy broth (TSB), followed by incubation for 18 h at 37°C. To establish the bacterial biofilms, 1 mL of the culture was transferred to fresh 9-mL TSB tubes, a stainless steel coupon was added to each, and the tubes were incubated 5 h at 37°C. Before being added to test tubes containing the carcass rinse, the surfaces were washed briefly in 1% Micro cleaning solution, rinsed in distilled water, sonicated for 30 min, and air-dried. For negative controls, surfaces were added to tubes containing only TSB. Coupons with attached bacteria were removed from the tubes and incubated at room temperature for 2 or 3 h, in the presence or absence of ionization. Afterwards, 100 μL of sterile water was added to the coupon surface, the surface was swabbed, and the swab was streaked on aerobic plate count agar.

Identification of Resistant Bacteria

Identification of bacterial isolates was determined with an automated BIOLOG/Micronaut System [25]. The isolates were inoculated onto microplate panels containing 95 biochemical tests for identification [26]. After incubation of the microplates for specified time periods, plates were read at 590 nm on a plate reader [27]. Changes in optical density indicative of substrate utilization were determined by the BIOLOG software using default parameters, and the isolates were classified by Numerical Taxonomic Cluster Analysis.

Statistics

Results were compared for the bacterial cfu during two separate experiments including three trials each. The data for each trial were the means from triplicate tests. Significant differences between bacterial counts for ionized and non-ionized samples were determined by the Student's two-tailed t-test at $P < 0.05$ [28].

RESULTS AND DISCUSSION

Natural bacterial populations from poultry carcasses were collected, grown as biofilms on stainless steel coupons, and assessed for susceptibility to negative air ionization. In previous work, we developed methods to measure

TABLE 1. Effect of exposure to negative air ionization for 3 h on bacterial biofilms (Exp. 1)

Trial[A]	1	2	3	Average (mean)
No. of cfu[B]—ionized	2.4×10^3	1.2×10^4	2.4×10^3	5.6×10^3
No. of cfu[B]—not ionized	1.67×10^6	2.49×10^6	2.65×10^6	2.27×10^6
Temperature (°C)	18	21	19	19
Humidity[C]	85.9	84.6	84.2	84.9
Reduction efficiency[D] (%)	99.9	99.5	99.9	99.8
Log reduction[E]	3	2	3	3

[A]The data for each trial were the means for triplicate tests.
[B]cfu = colony forming units. All swab samples were culture positive.
[C]The humidity was measured inside the ionization chamber.
[D]The percentage of bacterial reduction efficiency was calculated by the formula: ([(mean number bacterial cfu not ionized) – (mean number bacterial cfu ionized)] × 100)/(mean number bacterial cfu not ionized).
[E]The treated samples were near or below the level of negative controls without bacterial inoculum outside the chamber, which were 67% culture positive and averaged log 3.6.

bacterial attachment and demonstrate biofilm development from a mixed population of bacteria [29]. Stainless steel, although susceptible to bacterial attachment, is the most frequently used material for construction of equipment and other surfaces used in the food processing industry. The object of the electrostatic space charge system is to reduce developing or preexisting biofilms by transferring a strong negative electrostatic charge to bacterial cells within biofilms grown on stainless steel surfaces.

The ESCS dramatically reduced levels of bacteria in every trial. Table 1 shows the results when the coupons were exposed to the ionization for 3 h. All of the swab samples taken from coupons were culture positive for bacteria, and the bacterial counts from the ionized surfaces were significantly less than for the non-ionized surfaces ($P < 0.01$). An average of 2.3 million cfu was collected from each coupon not exposed to the ionizer as compared to an average of 5,600 cfu on each of the ionizer-exposed coupons, a reduction efficiency of 99.8%. Similar levels were observed during each of the three trials. Figure 2 compares the log cfu of bacteria remaining on surfaces after treatment by negative air ionization for 3 h. The net reduction was greater than 3 log units. These results with biofilms are comparable to the reductions obtained by Seo et al. [14] with aerosolized SE. Holt et al. [2] exposed XLT-4 plates to air in a caged layer room with and without negative air ionization treatment to show reduction efficiency for airborne levels of *Salmonella enterica* serovar *enteritidis*. When exposed to negative air ionization, levels on the plates were reduced by 95%. It should be noted that our plated samples were dilutions of swab samples of mixed bacterial populations from stainless steel surfaces, not collections of airborne salmonellae, and cannot be directly compared with the results of the study by Holt et al. [2]. A study at Massachusetts Institute of Technology killed up to 94% of *Staphylococcus aureus* cells sprayed on treated surfaces that had been coated with various alkyl bromides [30]. Microbial air levels in a dental clinic were significantly reduced (by 40 to 50%) with an ionizing generator [10].

Table 2 shows similar results were obtained when inoculated coupons were exposed to the ionizer for 2 h. Under the same conditions as the above experiment, there was no significant difference in the bacterial cfu for either of the ionized samples after 2 h when compared with colony-forming units after ionization for 3 h ($P > 0.05$). The average reduction efficiency of 97.3% for 2 h of exposure was slightly less than the 99.8% reduction for 3 h of ionization exposure. In each experiment, ionization reduced cfu levels to near or less than that of negative controls or the natural contamination of the air. Negative controls without bacterial inoculum outside the chamber were 67% culture positive, and the average cfu was 4,100. The average cfu after 3 h of ionization was 5,610 (Table 1) and after 2 h was 2.12×10^4 (Table 2).

Exposure times were limited to 3 h to avoid dehydration of the sample inoculum on the coupons. The average temperature adjacent to the chamber was 21°C during the experiments, and

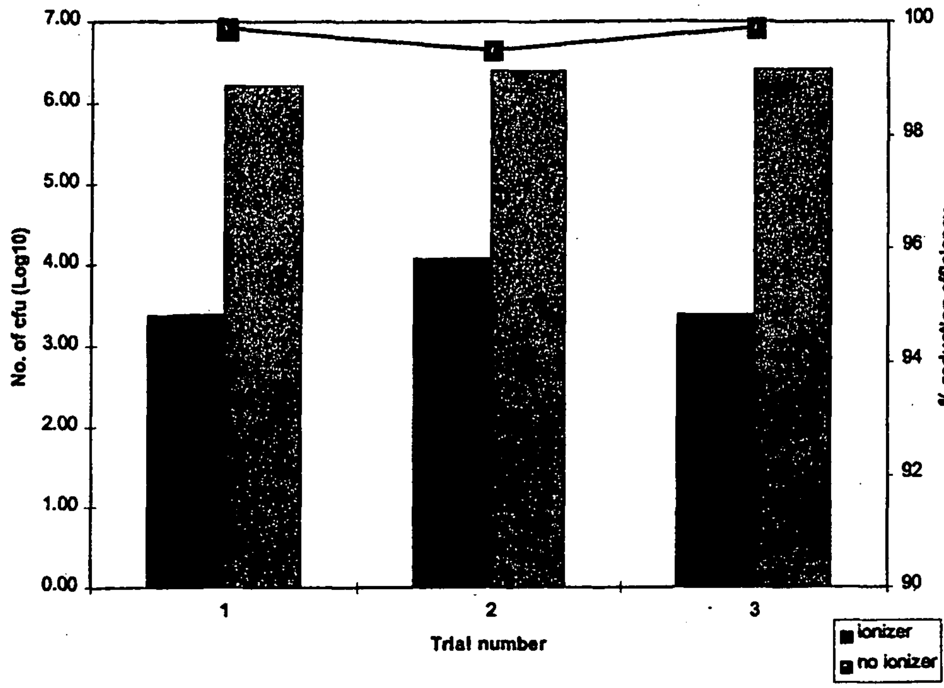

FIGURE 2. Comparison of colony-forming units (log10) remaining on ionized (dark bars) and non-ionized surfaces (light bars) after negative air ionization for 3 h, with reduction efficiency (■)—alternate axis).

the average relative humidity inside the chamber was 85%. Although the humidity differed slightly between the experiments (Tables 1 and 2), the humidity inside the chamber was controlled, and there was not a significant difference between bacterial counts of control samples ($P > 0.05$).

The morphological characteristics of the individual colonies on the plates not exposed to the ionizer presented many variations in size, configuration, margin, and elevation, typical for a mixed population of many species of bacteria (Figure 3). However, all the colonies on the plates exposed to the ionizer (less than 10 per plate for the samples ionized for 3 h) were round, with smooth margins, and convex elevations, and were less than 1 mm in diameter. The common size and shape of the few remaining colonies indicated that these bacteria were more resistant to ionization than the general popula-

TABLE 2. Effect of exposure to negative air ionization for 2 h on bacterial biofilms (Exp. 2)

Trial[A]	1	2	3	Average (mean)
No. of cfu[B]—ionized	4.9×10^4	4.9×10^3	9.7×10^3	2.1×10^4
No. of cfu[B]—not ionized	1.9×10^6	5.5×10^4	4.4×10^5	8.0×10^5
Temperature (°C)	23	21	21	21
Humidity[C]	86.2	83.4	84.9	84.8
Reduction efficiency[D] (%)	97.4	91.0	97.8	97.6
Log reduction[E]	2	1	2	2

[A]The data for each trial were the means for triplicate tests.
[B]cfu = colony forming units. All swab samples were culture positive.
[C]The humidity was measured inside the ionization chamber.
[D]The percentage of bacterial reduction efficiency was calculated by the formula: ([(mean number bacterial cfu not ionized) − (mean number bacterial cfu ionized)] × 100)/(mean number bacterial cfu not ionized).
[E]The treated samples were near or below the level of negative controls without bacterial inoculum outside the chamber, which were 67% culture positive and averaged log 3.6.

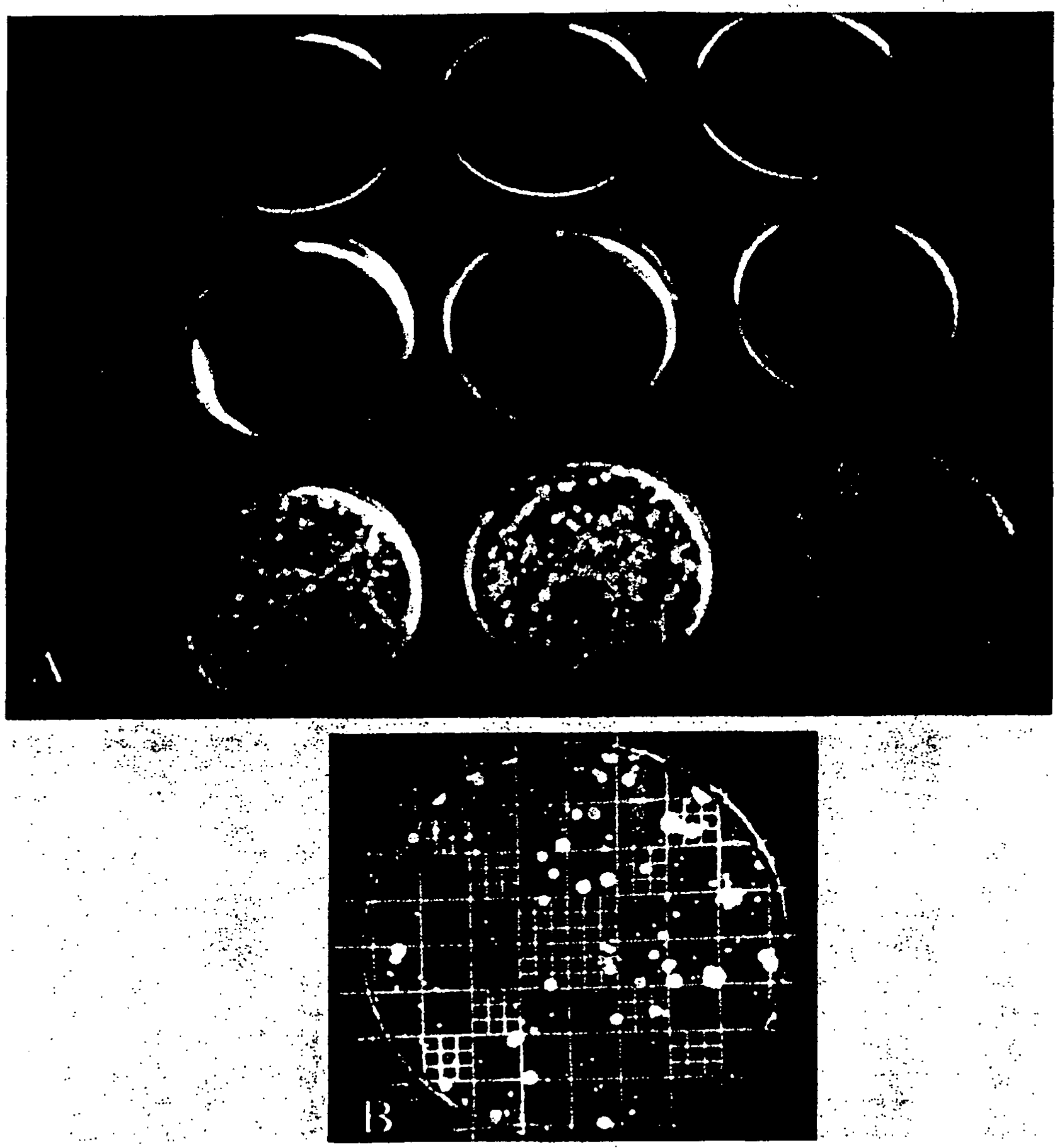

FIGURE 3. Typical colony morphology on plates after a 3-h ionization experiment: (A, top row) ionized bacterial sample, (A, middle row) open air control without bacterial sample, and (A, bottom row) non-ionized bacterial sample. A closeup of the non-ionized bacterial sample (B) shows the many variations in size and shape of the colonies from the bacterial sample.

tion of bacteria. Culturing these colonies yielded two species of bacteria that showed resistance to killing by negative air ionization. They were identified by the BIOLOG bacterial identification system [31] as *Enterococcus faecalis* and *Brochothrix thermosphacta*. Both are Gram-positive organisms, facultative anaerobes, and type species for their genus. *E. faecalis* occurs widely in the environment, particularly in feces of vertebrates. *B. thermosphacta* occurs mainly in meat products but is also widely distributed in the environment. Neither species is generally considered pathogenic [32].

Further study of these resistant bacteria may help determine the mode of action of the ESCS and help improve its efficiency in the processing environment. The mechanisms by which negative air ionization causes death of bacteria are not yet known. Changes in ionization can bring changes in catalysis, desorption,

and even enzymatic changes in chemical structure, affecting the surface and the bacterial cells [33, 34].

The proposed approach to reduction of bacterial contamination uses an ESCS, which has been shown, on a larger scale, to reduce airborne particulates and bacteria by charging the particles and collecting them on special grounded collectors or on walls and floors. A little-known effect of a strong electrostatic space charge is the ability to very effectively kill airborne and surface bacteria. These preliminary laboratory tests suggest that the effects are substantial and reproducible. Our results indicate that negative air ionization could potentially have an impact on the microbial load in a poultry processing facility, and at least a portion of this effect would be through direct killing of the organisms. The economic feasibility of using this technology in a poultry processing facility remains to be determined, but, if shown to be effective on a large scale, the basic equipment cost would probably be comparable to existing disinfection equipment and chemicals.

CONCLUSIONS AND APPLICATIONS

1. Negative air ionization dramatically reduced bacterial contamination on stainless steel surfaces.
2. Negative air ionization could potentially have a significant effect on the microbial load in a poultry processing facility.
3. This technology could have applications that extend into other food processing areas, medical institutions, and the home.

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